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(54) Title: **TEMPERATURE SENSITIVE POLYMERS**

(57) Abstract: The present invention relates to compositions comprising polymers whose solubility characteristics can be changed by incubation. Another aspect of this invention is the application of such temperature sensitive polymers as release systems of biologically active compounds. The polymers of the present invention comprise monomers which have modifiable functionality. The functionality of the monomers can for example be modified by the presence of hydrolysable groups. The modification is effected by the incubation, leading to a change of the water solubility characteristics of the polymer. The polymers used in the present invention contain hydrolysable chemical groups. As a result, the polymer's solution characteristics, specifically its lower critical solution temperature (LCST), change upon incubation.

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Title: Temperature sensitive polymers

The invention relates to compositions comprising polymers whose solubility characteristics can be changed by incubation. Another aspect of this invention is the application of such temperature sensitive polymers as release systems of biologically active compounds.

5 The fast developments in the field of molecular biology and biotechnology have made it possible to produce a large number of pharmaceutically interesting products in large quantities. For instance, pharmaceutically active peptides and proteins can suitably be used as drugs in the treatment of life-threatening diseases, *e.g.* cancer, and of several types of
10 viral, bacterial and parasital diseases; in the treatment of *e.g.* diabetes; in vaccines, *e.g.* for prophylactic aims; and for anti-conception purposes. Especially the specialized biological activities of these types of drugs provide tremendous advantages over other types of pharmaceuticals. Also low molecular weight pharmaceuticals, such as cytostatics, antibiotics, *etc.*, can be produced
15 in large amounts.

To illustrate the fast developments, it has been reported (see *e.g.* Soeterboek and Verheggen, Pharm. Weekblad 130 (1995) 670-675) that in the United States of America, about 275 biotechnological products are in phase IV studies, while more than 500 products are under investigation.

20 Examples of (recombinant) proteins, which are considered very interesting from a pharmacological point of view, are cytokines, such as interleukines, interferons, tumor necrosis factor (TNF), insulin, proteins for use in vaccines, and growth hormones.

Due to their nature, proteins and proteinaceous products, including
25 peptides, which group of products will be referred to as protein drugs herein-below, cannot be administered orally. These products tend to degrade rapidly in the gastro-intestinal tract, in particular because of the acidic environment and the presence of proteolytic enzymes therein.

Moreover, to a high extent protein drugs are not able to pass endothelial
30 and epithelial barriers, due to their size and, generally, polar character.

For these reasons, protein drugs have to be brought in the system parenterally, *i.e.* by injection, however, the pharmacokinetical profile of these products is such that injection of the product *per se* requires a frequent

administration. For, it is a known fact that proteinaceous material is eliminated from the blood circulation within minutes.

In other words, since protein drugs are chemically and/or physically unstable and generally have a short half-life in the human or animal body, multiple daily injections or continuous infusions are required for the protein drug to have a desired therapeutic effect. It will be evident that this is inconvenient for patients requiring these protein drugs. Furthermore, this type of application often requires hospitalization and has logistic drawbacks.

In addition, it appears that at least for certain classes of pharmaceutical proteins, such as cytokines which are presently used in *e.g.* cancer treatments, the therapeutic efficacy is strongly dependent on effective delivery, *e.g.* intra- or peritumoral. In such cases, the protein drugs should be directed to the sites where their activity is needed during a prolonged period of time.

Hence, there is a need for delivery systems which have the capacity for controlled release. In the art, delivery systems comprising soluble polymers have been proposed. Such delivery systems can be obtained by using such soluble polymers for example in the form of microparticles in which the protein drug is encapsulated. The polymer can be present throughout each microparticle, with the protein drug captured within the different polymer molecules. Alternatively, the polymer forms the outer membrane of the microparticle which contains the protein drug. However, *in vitro* or *in vivo* application of such systems have some inherent drawbacks. First, organic solvents have to be used to encapsulate proteins in the microparticles. Second, acidic products are frequently formed during degradation, which might result in a lowering of the pH. Both a low pH and organic solvents can affect protein stability. Furthermore, it appears to be difficult to control the protein release from these systems, which can lead to a burst release.

The present inventors have now found that the use of temperature sensitive polymers, and especially those with a lower critical solution temperature, have a number of advantages.

Temperature sensitive polymers with a lower critical solution temperature (LCST) are remarkable materials, in that below this temperature such polymers are soluble, and above it they precipitate. The lower critical solution temperature can be defined as the temperature at the point of inflection in a graph representing the amount of solids in the sample (for example as measured using light scattering techniques) vs. temperature.

Alternatively, the LCST can be defined as the lowest temperature where precipitated polymer particles are detected (the 'onset' temperature). An example of a light scattering curve is shown in Figure 1. Both the temperature at the point of inflection and the onset temperature are marked.

5 LCST-polymers can be used advantageously as drug release systems, because their preparation can be carried out at a temperature which is lower than the temperature at which the release is to be effected, for example the body temperature. Since the temperature can be kept low, there is little risk of denaturation or degradation of the (protein) drug to be released. Another
10 important advantage of the use of LCST-polymers in drug release systems is that the loading of the drug delivery system can be accomplished in an aqueous system, avoiding the use of toxic organic solvents. In addition, the LCST-polymers can be chosen such that they are degradable and/or can easily be excreted by the kidneys, once in soluble form.

15 The use of LCST polymers as controlled release systems is *e.g.* known from US-A-5 720 976. In this publication release systems are disclosed, wherein an active ingredient is encapsulated in liposomes. LCST polymers are grafted to the surface of liposomes. By choosing the ratio of respective monomers in the LCST polymers, the LCST value of the polymers can be
20 adjusted.

Furthermore, WO-A-92/07881 discloses that the solubility of polyacrylamide changes as a result of the presence of amide groups, which groups have a buffering effect. This pertains to the solubility *per se*, not to the LCST, which is not mentioned in this publication.

25 Also in EP-A-0 693 508 and in DE-A-4 023 578, it is described that the temperature sensitivity of certain polymers can be influenced by varying the ratio of the comonomers present in these certain polymers.

None of these prior art documents teach or suggest however, that LCST polymer systems can be modified, as is done in accordance with the present
30 invention in such a way, that the LCST value of the polymers changes during incubation and as a result of incubation, and by which the above mentioned advantages of the present invention can be obtained.

The LCST-polymer systems of the present invention can be used for drugs-targeting by incorporation into the matrix of compounds which make the
35 system suitable for physico-chemical or physical homing strategies. Such strategies employ a homing device, which is a characteristic protrusion on a

particle, capable of recognizing the target cell or tissue. Examples of such homing devices are monoclonal antibodies or fragments thereof, growth factor, insuline, sugar moieties, transferin, *etc.* In the physico-chemical strategy, homing devices are designed such that they only recognize and interact with specific structures on the surface of target cells or tissues. In the physical strategy, homing devices are designed to accumulate at target sites by physical means, such as a local magnetic field or heat. See for example D.J.A. Crommelin *et al.*, Adv. Drug. Deliv. Rev. 17 (1995) pp. 49-60.

The protein drug delivery systems based on LCST-polymers can be prepared conveniently by introduction of the protein drug into the polymer matrix. This is obtained by mixing the protein drug with the polymer, which is in dissolved state, for example because it is below its LCST. Subsequently, the mixture is brought in a state in which the polymer precipitates, for example by bringing it above its LCST, by which process the protein drug is captured within the precipitating polymer matrix, thus yielding a drug delivery system.

For the use in protein drug delivery systems, it is essential that the LCST-polymer to be applied is not below or above its critical solubility temperature. Effective application as controlled release system can only be obtained when the *in vivo* temperature is just below the critical solution temperature. Although it is known in the art - see e.g. the above discussed publications - that LCST-polymers can be modified by changing their composition, it will be clear that a choice with respect to the LCST has to be made prior to the administration. Once a certain polymer is chosen, its LCST is fixed. Variations of the application temperatures, as can occur easily for example as a result of differences or variations in body temperature, can lead consequently to different and non-gradual release profiles.

The present invention provides a polymer that is suitable for use in a controlled release system. Consequently, this polymer can be applied as a controlled release system having all the aforementioned advantages.

The present inventors have found that when certain water soluble polymers are chemically modified, their critical solution temperature will vary *in situ*, viz. upon *in vivo* or *in vitro* application in an aqueous environment. These changes are time dependent. In this description and the appending claims, application in an aqueous environment, under conditions enabling the reactions that result in the change of critical temperature, for example as a

result of hydrolysis, is referred to as incubation. It is also possible that the incubation is effected by enzymes present in the aqueous environment.

The polymer of the present invention comprises monomers which have modifiable functionality. The functionality of the monomers can for example be modified by the presence of hydrolysable groups. The modification is effected by the incubation, leading to a change of the water solubility characteristics of the polymer.

When reference is made to a polymer in this description, also copolymers, terpolymers and other interpolymers are to be understood. In fact, copolymers and terpolymers have the additional advantage that they provide an extra parameter affecting the final result, since different monomers, having different solubility characteristics, can be incorporated in one polymer, as to adjust the solubility characteristics (such as the solubility itself or the temperature dependency of the solubility) of the resulting copolymer. Copolymers and terpolymers thus form a preferred embodiment of the present invention.

The polymer according to the present invention is obtained by choosing the properties of the monomers such that upon incubation the functionality of the monomers changes and as a result the solubility and/or the temperature dependency of the solubility of the entire polymer, changes.

In a particular embodiment, the monomers are chosen so that their hydrophilicity changes upon incubation. As a result, the hydrophilicity of the entire polymer will change upon incubation. This will lead to a polymer with a different solubility and/or temperature dependency of the solubility.

More specifically, a temperature sensitive polymer can be obtained according to the present invention by choosing a monomer that is suitable for the envisaged application, e.g. a monomer that forms a pharmaceutically acceptable polymer. Suitable monomers are the monomers selected from the group comprising ethylene glycol, lactic acid, acrylamide, methacrylamide, acrylic acid, and derivatives and substituted species thereof. These monomers and/or other monomers are then reacted under suitable conditions to form homopolymers of one of these monomers or copolymers, terpolymers or other polymers of two or more monomers. Preferred monomers are *N*-isopropyl acrylamide (NIPAAm), 2-hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), acrylamide (AAM), glyceryl methacrylate or glycidyl methacrylate (GMA), glyceryl acrylate or glycidyl acrylate (GA), hydroxypropyl

methacrylamide (HPMAAm), dimethyl-aminoethyl methacrylate (DMAEMA) and dimethylaminoethyl acrylate (DMAEA).

In a preferred embodiment of the present invention, the change of solubility characteristics is effected by hydrolysis of a group present on at least one of the monomers that form the polymer. Such a group is preferably chosen from ester, amide, carbonate, carbamate, and anhydride groups. Even more preferably such a group comprises a lactate unit, such as a monolactate, a dilactate or an oligolactate group. In case of *in vivo* application such a group can advantageously be an enzymatically or chemically hydrolyzable group. The ester groups are introduced in the polymer by choosing suitable monomers as a starting material, such as 2-hydroxyethyl methacrylate-monolactate. The monomers can be provided with ester groups by techniques known to the person skilled in the art.

The polymer can be synthesized by starting from a mixture of the monomers and carrying out the polymerization reaction. It is also possible to first produce the polymer and subsequently functionalize it by adding suitable groups. Compositions according to the present invention comprise block copolymers or terpolymers, random copolymers or terpolymers, random copolymers and polymeric networks, all of which polymers can be grafted, and mixtures thereof.

The solubility characteristics of the compositions according to the present invention will change upon incubation, for example when contacted with aqueous media, such as will be the case in *in vivo* application. When the polymers used are of the type that displays a lower critical solution temperature, this critical temperature is preferably between 0 to 100°C before incubation to form a polymer having a critical solution temperature that is within the same range.

For application in mammals, the polymers according to the present invention have a critical temperature for both the composition as synthesized and the composition after incubation which is around body temperature, *viz.* between about 20 to 45°C, preferably between 30 and 42°C, and most preferably between 36 and 38°C. However, more preferably the value of LCST crosses the normal human body temperature (which is typically 37°C) upon incubation so that the LCST before incubation is below 37°C, preferably below 35°C, and LCST after incubation is above 37°C, preferably above 38°C.

A preferred embodiment of the present invention is the use of the polymer in or as a controlled release system. For example for the controlled administration of drugs, such as protein drugs.

The controlled release system of the present invention can be used for
5 the release of biologically active compounds, such as pharmaceutic compounds, *e.g.* pharmaceutically active peptides and proteins, genetic material *e.g.* nucleotides, plasmid DNA, anti-sense oligonucleotides, nutrients, *etc.*

When the system is used for the delivery of genetic material, *e.g.* the delivery of plasmid DNA or anti-sense oligonucleotides, the LCST polymer
10 preferably comprises a cationic group, such as DMAEMA.

It is also possible to make the controlled release systems which can be obtained by the present invention in the form of polymeric micelles. Polymeric micelles can be formed by the synthesis of amphiphilic blockcopolymers, *e.g.* AB block copolymers of PEG and poly(β -benzyl-L-aspartic acid) (G.S. Kwon, M. Naito, M. Yokoyama, T. Okana, Y. Sakurai and K. Kataoka, Pharm. Res. 12
15 (1995) pp. 192-195). In aqueous solutions, these polymers form micelles with a size of around 20 nm (G.S. Kwon, M. Naito, M. Yokoyama, T. Okana, Y. Sakurai and K. Kataoka, Langmuir, 9 (1993) pp. 945-949). The hydrophobic core of these micelles can be loaded with drugs, *e.g.* the anti-cancer agent
20 adriamycin. After *in vivo* administration of the these systems the adriamycin loaded micelles selectively accumulate in certain tumors, simultaneously releasing the drug, which results in killing of tumor cells (M. Yokoyama, S. Fukushima, R. Uehara, K. Okamoto, K. Kataoka, Y. Sakurai and T. Okano, Journal of Controlled Release, 50 (1998) pp. 79-92).

25 Polymers with an LCST have also been applied to design polymeric micelles. Below the LCST, the thermosensitive polymer acts as hydrophilic part of the system (*e.g.* in AB blockcopolymers of *N*-isopropyl acrylamide and styrene; S. Cammas, K. Suzuki, C. Sone, Y. Sakurai, K. Kataoka, and T. Okano, Journal of Controlled Release, 48 (1997) pp. 157-164). Also, systems
30 have been described in which PNIPAAm forms the hydrophobic part of the polymeric micelle (in block copolymers of poly(ethylene glycol)) and poly(*N*-isopropylacrylamide; M.D.C. Topp, P.J. Dijkstra, H. Talsma and J. Feijen, Macromolecules, 30 (1997) pp. 8518-8520). After administration of the drug loaded PNIPAAm systems and arrival at the target site, drug release can then
35 be triggered by local hypothermia. Hypothermia is, however, not easily done or

technically feasible for all tissues and organs, which limits the applicability of these systems.

These disadvantages can be overcome by using polymers composed of a hydrophilic block covalently linked to a block composed of thermosensitive polymer with hydrolyzable side groups. Such a hydrophilic block preferably comprises poly(ethyleneglycol) (PEG). When the LCST of the thermosensitive block is initially below body temperature, polymeric micelles are formed at 37°C. Due to hydrolysis of the side groups present in the thermosensitive block of the system, the LCST will increase, resulting in destabilization of the micelle when the LCST passes 37°C. When a drug is incorporated in the hydrophobic core, its release will be affected by this process. These systems can be favorably applied in *e.g.* cancer treatment, treatment of rheumatism, arthritis, infections and/or inflammations.

As mentioned above, the polymers of the present invention comprise all possible polymer architectures, such as (multi-)block copolymers (such as AB, ABA, ABAB, *etc.*) or graft copolymers, random copolymers or terpolymers, or a polymeric networks; all of which may be grafted.

AB blockcopolymers with a thermosensitive block A (*e.g.* NIPAAm copolymerized with a comonomer with hydrolyzable side groups) and a watersoluble B block (*e.g.* PEG) that form micelles when the LCST is passed, can be obtained by any known technique in the art for making AB blockcopolymers. Conveniently, these polymers are prepared using a so called macroinitiator.

A macroinitiator is a macromolecular initiator that is formed *e.g.* by coupling a low molecular weight initiator, such as 4,4'-azobis(4-cyanopentanoic acid), (HO-CO-CH₂.CH₂.C(CH₃)(CN)-N=)₂ (ABCPA), via its carboxyl groups at to the terminal OH group of a methoxylated compound such as PEG (*i.e.* CH₃-O-PEG-OH). In this way a compound of the formula (CH₃-O-PEG)₂-ABCPA is formed. Typically, PEG with a Mw of about 1500-6000, is used for this purpose. Preferably PEG with a Mw of about 5000 (PEG 5000) is used to form a (PEG 5000)₂-ABCPA macroinitiator. When this initiator decomposes by heat, a PEG chain with one radical is formed. This radical subsequently initiates the polymerization of monomers (such as NIPAAm/HPMA-lactate, as described hereinbelow), by which an AB block copolymer is formed. In aqueous solution such polymers form a micellar structure when the temperature rises above its LCST. These micelles destabilize when the hydrolysis results in a A block with

an increased LCST (above the temperature at which the micelles are applied, preferably at body temperature).

For a NIPAAm / HPMAM-lactate copolymers of the invention the ratio of NIPAAm / HPMAM-lactate is preferably from 5-80, most preferably from 20-50.

Alternatively, ABA block copolymers may be synthesized via the macroinitiator route by using instead of a monofunctional (*i.e.* α -methoxy) PEG or equivalent thereof, an α - ω -hydroxyl derived macroinitiator, *viz.* a macroinitiator which has the ABCPA-groups at both ends of the molecule. When this initiator decomposes by heat, a PEG chain with two radicals is formed. These radicals subsequently initiate the polymerization of monomers (such as NIPAAm/HPMA-lactate), by which an ABA block copolymer is formed. The ABA block copolymers formed by this route will be soluble in water below the LCST. When the temperature is risen above the LCST of block A, a phase separated system will be formed, wherein as a result of the choice of block copolymer architecture, a hydrogel will be obtained. This hydrogel will dissolve gradually when the LCST of block A increases to above 37°C, due to the hydrolysis of the groups present on the monomers of this block. These systems are especially suitable for immobilizing cells, which can be employed in biotechnology and tissue engineering. Like the other systems mentioned hereinabove, these macroinitiator systems can also be used as matrix for controlled release of active ingredients, in particular pharmaceutical proteins.

It is noted, however, that also the ABA block copolymers – like the AB block copolymers – may be prepared by other, conventional synthesis routes as well.

When the polymers of the present invention are used for targeting drug purposes, the release system is made of particles which particles have an average diameter of less than 1 μ m, preferably less than 100 nm. To be of practical value, these particles will usually have to be larger than several nm, *e.g.* greater than 10 nm.

The polymer used in the present invention is for example a terpolymer of *N*-isopropylacrylamide (NIPAAm), HEMA-monolactate and acrylamide (AAm) in which the respective NIPAAm/HEMA-monolactate and AAm monomer ratios are chosen to be for example 50/20/30. It will be understood that the ratio of different monomers which constitute the

copolymer or terpolymer, will influence the LCST and its development upon incubation. Generally for practical application, e.g. application in mammals, it is desirable to choose the ratios such that the LCST before incubation is below body temperature and after incubation above body temperature. The optimal
5 ratio of each of the monomers will consequently depend strongly on the materials used and the envisaged application. The optimal values can be determined experimentally, as will be illustrated in the Examples hereinafter.

An important aspect of the present invention is the use of hydrolysable chemical groups in a temperature sensitive polymer in order to
10 change said polymer's solution characteristics, specifically its critical solution temperature, more specifically its lower critical solution temperature (LCST).

It will be understood that apart from changing the solubility of polymers having a lower critical solution temperature, this can also be applied to polymers having a higher critical solution temperature, viz. polymers which
15 dissolve at temperatures higher than their critical temperature, and precipitate at temperatures lower than this critical temperature.

The effect of the incubation can be an increase as well as a decrease of the critical temperature upon incubation.

The controlled release systems of the present invention can be
20 prepared by the synthesis of a water soluble polymer. This is done by a) functionalizing a monomer with hydrolysable groups, b) mixing of said monomer with at least one monomer of a different type in a suitable ratio using a suitable solvent in the presence of an initiator and/or a catalyst to form said polymer c) removing said solvent and dissolving the polymer, and d)
25 precipitating said polymer; in which process the functionalizing of the monomers of step a) is optionally carried out after step b) on the monomers as they are present in the polymer; and subsequently mixing said water soluble polymer with a releasable compound.

Other suitable initiators as well as the catalysts for step a) are known
30 in the art. An example of a suitable initiator is α, α' -azoisobutyronitrile (AIBN). An example of a suitable catalyst is stannous octoate (SnOct_2).

Apart from application as a controlled release agent, the polymers of the present invention can be applied as release systems for a variety of compounds in different applications, such as enzymes, colorants or other
35 additives in laundry applications, adhesives in glues, insecticides or nutrients in agricultural applications, etc. Further possible applications are the topical

administration polymers of the present invention loaded with active ingredients, *e.g.* for the treatment of burns. The polymers of the invention can also be used for the delivery of genetic material (DNA delivery).

The present invention will now be illustrated in the following
5 Examples.

EXAMPLE 1

Synthesis of poly(*N*-isopropylacrylamide-co-2-hydroxyethyl 10 methacrylate-monolactate)

2-(Methacryloyloxy)ethyl-mono- or -oligolactate, which is HEMA esterified with one or more lactic acid groups, was synthesized using HEMA and L-lactide at a molar ratio of 2 to 1, essentially as described in Van Dijk-
15 Wolthuis, W.N.E., Tsang, S.K.Y., Kettenes-van den Bosch, J.J. and Hennink W.E., 'A New Class of Polymerizable Dextrans with Hydrolyzable Groups: Hydroxyethyl Methacrylated Dextran With and Without Oligolactate Spacer', Polymer, 38, 6235-6242, (1997).

A mixture of HEMA (13.02 gram; 100 mmol) and L-lactide (7.2 gram,
20 50 mmol) was stirred at 110°C in a nitrogen atmosphere until the lactide was molten. Next, a catalytic amount of SnOct₂ (0.4 g, 1 mol-% with respect to HEMA; diluted 1:1 with toluene) was added and the reaction mixture was stirred for 1 hour. After one hour, the reaction mixture was allowed to cool to room temperature and insoluble products, probably consisting of SnOct₂
25 complexes, were removed by centrifugation (16000 G; 5 minutes). Next, 1 g of the clear, viscous mixture was dissolved in 1 cm³ acetonitrile and 500 µl of this solution was injected onto a preparative HPLC column (Econospher C8, 10 µm, 250x22 mm; Alltech, IL). An Äcta™ Purifier system (10XT, Pharmacia Biotech, Sweden) was used. Elution was done using a mobile phase consisting
30 of 59% (w/w) water and 41 % (w/w) acetonitrile with flow rate of 5 cm³/min (UV detection λ=254 nm). The chromatograms were analyzed with Unicorn 2.30 software (Pharmacia Biotech, Sweden). Under these conditions HEMA, 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)ethyl-di-lactate had a retention time of approximately 16, 19 and 24 minutes, respectively.

Corresponding fractions of different runs were collected, pooled and freeze dried. The obtained products were characterized by ^1H -NMR (see Figure 3).

^1H -NMR 2-(Methacryloyloxy)ethyl-lactate (Figure 3A; CDCl_3): δ 6.11 (s, 1H, H_a), 5.59 (s, 1H, H_a'), 4.50-4.26 (m, 4H, H_c , H_d), 4.42 (q, $J_{gh} = 6.9$ Hz, 1H, H_g), 2.80 (bs, OH_i), 1.94 (s, 3H, H_b), 1.40 (d, $J_{gh} = 6.9$ Hz, 3H, H_h).

^1H -NMR 2-(Methacryloyloxy)ethyl-di-lactate (Figure 3B; CDCl_3): δ 6.11 (s, 1H, H_a), 5.59 (s, 1H, H_a'), 5.19 (q, 1H, H_e), 4.50-4.26 (m, 4H, H_c , H_d), 4.42 (q, $J_{gh} = 6.9$ Hz, 1H, H_g), 2.80 (bs, OH_i), 1.94 (s, 3H, H_b), 1.55 (d, 3H, H_f), 1.45 (d, $J_{gh} = 6.9$ Hz, 3H, H_h).

Mixtures of *N*-isopropylacrylamide (NIPAAm) and HEMA-monolactate were prepared with NIPAAm/HEMA-monolactate ratios of 100/0, 95/5, 90/10, 80/20, 65/35 and 50/50 (mol/mol), the total monomer concentration being 0.1 g/cm³ in 1,4-dioxane. α, α' -azoisobutyronitril (AIBN) was added as initiator in an amount so that monomer/initiator = 250/1. The copolymerization was conducted at 60°C for 20 hours in a nitrogen atmosphere. Subsequently, the solvent was removed under pressure and the copolymers were dissolved in acetone (20 % (w/v)) and precipitated in an excess of diethylether. The precipitated polymers were isolated by filtration and dried in a vacuum oven at 40°C.

REFERENCE EXAMPLE 1

Synthesis of poly(*N*-isopropylacrylamide-co-2-hydroxyethyl methacrylate)

Polymers without the monolactate group on the HEMA monomer, *i.e.* poly(*N*-isopropylacrylamide-co-2-hydroxyethyl methacrylate), were prepared to be used as a reference, starting from NIPAAm and 2-hydroxyethyl methacrylate (HEMA) in ratios NIPAAm/HEMA of 100/0, 95/5, 90/10, 80/20 and 60/40. The synthesis was carried out as in Example 1.

EXAMPLE 2**Hydrolysis of poly(*N*-isopropylacrylamide-co-2-hydroxyethyl methacrylate-monolactate)**

5 The different polymers from Example 1 were dissolved in phosphate buffered saline (PBS, pH = 7.2) in concentrations of 5 mg/cm³ at a temperature below the LCST. The pH of the homogeneous solution was adjusted to 10.5 by addition of 0,1 N NaOH. The samples were incubated at 37°C, which resulted in precipitation of the polymer. After three days, the samples were cooled to
10 room temperature and the pH was adjusted to 7.2 by addition of 0.1 N HCl. Next, the samples were filtrated (FP 030/3, Disposable Filter Holder, 0.2 mm; Schleicher & Schuell GmbH, Dassel, Germany) and then dialyzed against water at 4°C (Dialysis Tubing-Visking, Size 9 Inf Dia 36/32"-28.6 mm: 30 M, MWCO-12-14000 Daltons; MEDICELL International Ltd., London, Great
15 Britain). The hydrolyzed polymers were collected after freeze-drying.

 The LCST of the different polymers was determined by static light scattering. The degree of light scattering at 650±5 nm was measured under a 90° angle, using a Luminescence Spectrometer LS50 (Perkin Elmer Limited, United Kingdom). Samples were prepared by dissolving the copolymers in PBS
20 (pH = 7.2) in a concentration of 0.1 mg/cm³. The temperature of the samples was increased from 10 to 40°C at a rate of 1°C/min. The intensity of the scattered light was measured at intervals of 0.2°C. Inflection points of the intensity-temperature curves were taken as the LCST values, a typical example is given in Figure 1.

25 The LCST values for the poly(NIPAAm-co-HEMA-monolactate) before and after incubation are given in Figure 2, together with the LCST values of the reference polymer. From Figure 2 it follows that the LCST of poly(NIPAAm-co-HEMA-monolactate) upon incubation increases due to the hydrolysis of the lactate groups. The LCST values for the different
30 poly(NIPAAm-co-HEMA-monolactate) copolymers after incubation approach that of the poly(NIPAAm-co-HEMA), which lacks the lactate group. This increase of LCST is the result of the hydrolysis of the lactate groups.

EXAMPLE 3**Synthesis of terpolymers of NIPAAm/
HEMA monolactate/acrylamide**

5 Mixtures of *N*-isopropylacrylamide (NIPAAm), HEMA-monolactate and acrylamide (AAm) were prepared with NIPAAm/HEMA-monolactate and AAm ratios of 70/20/10, 60/20/10/ and 50/20/30 (mol/mol/mol), the total monomer concentration being 0.1 g/cm³ in 1,4-dioxane (volume 5 ml). AIBN was used as initiator (250 mol monomers/1 mol initiator). The
10 copolymerization was conducted at 60°C for 20 hours in a nitrogen atmosphere.

 The terpolymer prepared with the 70/20/10 comonomer ratio was isolated as described for the NIPAAm/HEMA-monolactate polymers (Example 1). The other terpolymers were isolated as follows. To the polymer solution, 20
15 ml water was added and the resulting mixture was dialyzed against water at 4°C. The polymers were collected after freeze-drying.

REFERENCE EXAMPLE 3**Synthesis of NIPAAm/HEMA/AAm terpolymers**

20 Polymers without the monolactate group on the HEMA monomer, *i.e.* NIPAAm/HEMA/AAm terpolymers, were prepared to be used as a reference, starting from NIPAAm, HEMA and AAm in ratios NIPAAm/HEMA/AAm of 70/20/10, 60/20/20 and 50/20/30 (mol/mol/mol).

25 The synthesis was carried out as in Example 3 with a total monomer/AIBN ratio of 125/1 mol/mol). The polymers were isolated after dialysis and freeze drying as described for the polymers in Example 3.

30

EXAMPLE 4**Hydrolysis of NIPAAm/HEMA-monolactate/AAm terpolymers.**

 Hydrolysis of the lactate ester side groups of the different polymers from Example 3 was done using the procedure described in Example 2.

35 The LCST of the different polymer was determined by static light scattering as described in Example 2. Table 1 summarizes the results.

Table 1.

Terpolymer	feed ratio (mol/mol/mol)	LCST+S.D. (°C)+	LCST+S.D. (°C)++
NIPAAm/HEMA/AAm	70/20/10	28.7±0.5	31.9±0.5
NIPAAm/HEMA/AAm	60/20/20	36.7±0.3	40.6±0.5
NIPAAm/HEMA/AAm	50/20/30	42.3±0.3	52.6±0.5
NIPAAm/HEMA-monomolactate/AAm	70/20/10	24.0±0.1	26.7±0.1
NIPAAm/HEMA-monomolactate/AAm	60/20/20	30.7±0.5	35.0±0.9
NIPAAm/HEMA-monomolactate/AAm	50/20/30	39.2±0.3	43.6±0.4
NIPAAm/HEMA/AAm*	70/20/10	33.8±1.8	38.3±1.1
NIPAAm/HEMA/AAm*	60/20/20	59.2±0.3	64.8±0.8
NIPAAm/HEMA/AAm*	50/20/30		**

- 5
- *

after hydrolysis of the lactate ester side groups
- **

broad temperature range
- +

determined with static light scattering (n=3-4); onset point (see Figure 1)
- ++

determined with static light scattering (n=3-4); inflection (see Figure 1); S.D. is the standard deviation

10

Table 1 shows that it is possible to synthesize polymers that have an LCST below body temperature, but have an LCST above this temperature after hydrolysis of the side groups. This means that the polymer is initially insoluble in water at 37°C, however, the polymer gradually start to dissolve once (part of) the side groups are hydrolyzed.

15

EXAMPLE 5

Hydrolysis of poly(NIPAAm-co-glycidylmethacrylate)

20

Poly(NIPAAm-co-glycidylmethacrylate) was synthesized as in the previous Examples using different ratios of the NIPAAm and glycidylmethacrylate monomer. The epoxy group of the glycidylmethacrylate can be hydrolized to the corresponding diol group, yielding a

glycerylmethacrylate functional unit. Hydrolysis was performed using an aqueous solution of sulfuric acid. The results are summarized in Table 2.

5 **Table 2.**

NIPAAm/glycidylmethacrylate (mol/mol)	LCST \pm S.D. (°C) ⁺	LCST \pm S.D. (°C) ⁺⁺
95/5	29.1 \pm 0.4	30.4 \pm 1.5
90/10	27.3 \pm 0.2	32.6 \pm 0.5
80/20	25.1 \pm 0.3	34.2 \pm 1.1
65/35	20.2 \pm 0.1	35.9 \pm 1.4
50/50	18.4 \pm 0.4	41.9 \pm na*

+ before hydrolysis

++ after hydrolysis; S.D. is the standard deviation

* not available

10 From this Example it follows that the ratio of the monomers at which the resulting polymer has desirable LCST values can be determined experimentally.

15

EXAMPLE 6

Synthesis of N-(2-hydroxypropyl) methacrylamide-lactate

N-(2-hydroxypropyl) methacrylamide (HPMAm) esterified with lactate groups (HPAAm-lactate) was synthesized essentially as HEMA-lactate described in Example 1. A mixture of HPMAm (30 mmol) and L-lactide (30 mmol) was heated to 110°C. Subsequently, a catalytic amount of stannous octoate (SnOct₂, 1 mol-% with respect to HPMAm) was added. A polymerization inhibitor (4 methoxyphenol, 1 mol-% with respect to HPMAm) was added. The resulting mixture was stirred for one hour at 110°C and thereafter allowed to cool to room temperature.

20

25

Essentially pure HPMAM with one lactate group (HPMAM-(lactate)₁) and two lactate groups (HPMAM-(lactate)₂) were obtained from the crude reaction mixture by preparative column chromatography (Econosphere C8, 10 μ m, 250×22 mm, Alltech, IL) essentially as described for the
5 purification HEMA-lactate using an Acta™ purifier system in Example 1. Samples enriched in either HPMAM-(lactate)₁ or HPMAM-(lactate)₂ were obtained using column chromatography (straight phase; silica 60H). The polydisperse product was dissolved in dichloromethane and applied onto the column and the column was developed using dichloromethane with 2 %
10 methanol to obtain the enriched fractions.

The identity of the obtained fractions was determined by ¹H-NMR (as a solvent CDCl₃ was used).

The purity of the fractions was evaluated by HPLC (analytical column: LiChrosphere 100 RP-18 (5 μ m, 125 × 4 mm i.d.)), using acetonitrile /
15 water = 5 /95 (w/w) (eluent A) and acetonitrile / water = 95 / 5 (w/w) (solvent B). The gradient was run from 100 % A to 100 % B in 26 minutes, with the flow rate 1 ml/min. For detection was used UV-detector at the wavelength 210 nm.

20

EXAMPLE 7

Synthesis of poly(N-isopropylacrylamide-co-N-(2-hydroxypropyl) methacrylamide-lactate)

25 Copolymers of N-isopropylacrylamide (NIPAAm) and N-(2-hydroxypropyl) methacrylamide-lactate (HPMAM-lactate) were prepared by radical polymerization. HPMAM-lactate was synthesized as described in Example 6 and purified by column chromatography. The average length of the lactate graft (= Degree of Polymerization, DP) was 3 (11% DP1, 48% DP2, 10%
30 DP3, 24% DP4, the remaining fraction had DP >4). The NIPAAm / HPMAM-lactate ratios were 95/5, 90/10, 80/20, 65/35 and 50/50 (mol/mol). The total monomer concentration was 0.1 g/ml in 1,4-dioxane and α,α' -azoisobutyronitril

(AIBN) was used as initiator (total amount of monomers/initiator = 250/1 mol/mol). The copolymerization was conducted at 60°C for 20 h in a nitrogen atmosphere. Subsequently, the solvent was removed under reduced pressure and the copolymers were dissolved in acetone (around 20% (w/v)) and
5 precipitated in an excess of diethyl ether. The precipitated polymers were isolated by filtration and dried in a vacuum oven at 40°C.

REFERENCE EXAMPLE 7

10 Synthesis of poly(N-isopropylacrylamide-co-N-(2-hydroxypropyl) methacrylamide)

Poly(N-isopropylacrylamide-co-N-(2-hydroxypropyl) methacrylamide) copolymers were prepared starting from NIPAAm and N-(2-
15 hydroxypropyl) methacrylamide (HPMAm) in ratios NIPAAm/HPMAm of 95/5, 90/10, 80/20, 65/35 and 50/50 (mol/mol). The total monomer concentration was 0.1 g/ml in 1,4-dioxane and AIBN was used as initiator (total amount of monomers/initiator = 250/1 mol/mol). The copolymerization was conducted at 60°C for 20 h in a nitrogen atmosphere.

20 Polymers with the monomer ratio 95/5 and 90/10 were isolated as follows. The solvent was removed under reduced pressure and the copolymers were dissolved in acetone (around 20% (w/v)) and precipitated in an excess of diethyl ether. The precipitated polymers were isolated by filtration and dried in a vacuum oven at 40°C.

25 The polymers with the monomer ratios 80/20, 65/35 and 50/50 were isolated as follows. 20 ml of distilled water was added and the solutions were brought at 100°C and thereafter cooled at 0°C. The so formed clear and homogeneous solutions were extensively dialyzed against water. Thereafter, the polymers were obtained after lyophilization.

EXAMPLE 8**Hydrolysis of poly(N-isopropylacrylamide-co-N-(2-hydroxypropyl) methacrylamide-lactate)**

5 The different polymers of Example 6 were dissolved in phosphate buffered saline (PBS, pH = 7.2) in concentration of 5 mg/ml at a temperature below the LCST. The pH of the homogenous solutions was adjusted to ≈ 11 by addition of 1N NaOH. Next, the solutions were incubated at 37°C for 4-7 days. After 1 day a drop in pH was observed, indicating that lactic acid was split off
 10 from the polymer backbone. Next, the samples were cooled to $\approx 5^\circ\text{C}$ and then dialyzed against water at 4°C (Dialysis Tubing-Visking, Size 9 Inf Dia 36/32"-28.6 mm: 30M (Approx), MWCO-12-14000 Daltons; MEDICELL International LTD., London, Great Britain). The hydrolysed polymers were collected after freeze-drying. As a control, the different copolymers of reference Example 7
 15 were treated in the same way. The results are given in Table 3.

Table 3. The LCSTs of the poly (NIPAAm-co-HPMAm-lactate) copolymers before and after hydrolysis and of the poly(NIPAAm-co-HPMAm) copolymers before and after incubation at 37°C (pH ≈ 11).

Comono- mer ratio (mol/mol)	NIPAAm/ HPMAm-lactate		NIPAAm/ HPMAm-lactate (after hydrolysis)		NIPAAm/ HPMAm		NIPAAm/ HPMAm (after the hydrolysis treatment)	
	OS (°C)	IP (°C)	OS (°C)	IP (°C)	OS (°C)	IP (°C)	OS (°C)	IP (°C)
0	28.7 \pm 1.0	30.1 \pm 0.5	28.7 \pm 1.0	30.1 \pm 0.5	28.7 \pm 1.0	30.1 \pm 0.5	28.7 \pm 1.0	30.1 \pm 0.5
5	23.6 \pm 0.2	24.7 \pm 0.3	26.7 \pm 0.1	28.3 \pm 0.2	28.4 \pm 1.0	30.7 \pm 0.7	28.4 \pm 0.1	30.1 \pm 0.3
10	22.7 \pm 0.1	24.0 \pm 0.5	28.9 \pm 0.1	30.8 \pm 0.1	30.7 \pm 0.2	33.8 \pm 0.2	30.5 \pm 0.4	33.1 \pm 0.5
20	19.8 \pm 0.5	21.0 \pm 0.5	34.0 \pm 0.2	36.3 \pm 0.1	32.1 \pm 0.2	38.4	32.9 \pm 0.1	35.4 \pm 0.2
35	11.3 \pm 0.2	14.8 \pm 0.1	43.5 \pm 0.3	48.5 \pm 0.4	35.6 \pm 1.0	40.2 \pm 0.6	38.1 \pm 0.2	42.2 \pm 0.2
50	Could not be dissolved in PBS		N.D	N.D	45.0 \pm 3.0	52.8 \pm 1.6	47.2 \pm 1.9	

20 OS: onset
 IP: inflection point
 values \pm standard deviation (n=3-5)
 N.D. not determined

25 Table 3 shows that it is possible to synthesize polymers that have an LCST below body temperature, but have an LCST above this temperature

after hydrolysis of the side groups. This means that the polymer is initially insoluble in water at 37°C and dissolves once (part of) the side groups are hydrolyzed. From this Example it follows that in order to have the temperature dependence of this specific polymer (NIPAAm / HPMAM-lactate) tailored around 37°C, the ratio of NIPAAm / HPMAM-lactate should be from 5 to above 50, preferably from 20-50.

EXAMPLE 9

10 Degradation kinetics of 2-hydroxyethyl methacrylate- mono(di)lactate (HEMA-mono(di)lactate) at pH 7.5 in PBS

 The time required for an insoluble copolymer with hydrolyzable side groups to become soluble under physiological conditions depends on the copolymer composition and the hydrolysis kinetics of the side chain. Therefore, the degradation kinetics of different monomers was investigated.

 Stock solutions (10 mM, 10 ml) were prepared by dissolving HEMA-monolactate and HEMA-dilactate separately in DMSO. Next, 1 ml of stock solution was diluted with 9 ml 100 mM PBS (pH 7.5). The degradation was carried out in glass bottles (20 ml) in a water-bath at 37°C. Samples (300 µl) of these solutions were periodically drawn and diluted with 700 µl of 1M acetic buffer (pH 3.4) to stop further degradation. The degradation of HEMA-monolactate was followed for 5 days and the degradation of HEMA-dilactate was followed for 10 h.

25 The different samples were analyzed by HPLC (LiChrosphere 100 RP-18 (5 µm, 125 × 4 mm i.d.) to monitor the concentrations of HEMA-monolactate, HEMA-dilactate, HEMA and MAAC (methacrylic acid). For the analysis of HEMA-monolactate an isocratic system was used (methanol / water = 15 / 85 (w/w), pH 2 (adjusted by addition of perchloric acid). The flow rate was

1 ml/min. The injection volume was 200 μ l. For detection was used UV-detector at the wavelength 210 nm.

A gradient system was used for the analysis of HEMA-dilactate (methanol / water = 5 / 95 (w/w), pH 2 (eluent A) and methanol / water = 95 / 5 (w/w), pH 2 (eluent B). pH of both eluents was adjusted by addition of perchloric acid. The gradient was run from 100 % A to 100 % B in 26 minutes, with the flow rate 1 ml/min. For each degradation reaction, the reaction rate constant k_{obs} was calculated from the slope of the natural logarithm of the residual HEMA-monolactate or HEMA-dilactate versus time. The half-life is then calculated by $\ln 2 / k_{obs}$. Table 4 summarizes the results.

Table 4. Degradation at pH 7.5

Monomer			Major degradation products
	k_{obs} (s^{-1})	$t_{1/2}$ (h)	
HEMA-monolactate	6.1E-06	31.7	HEMA and lactic acid
HEMA-dilactate	6.1E-05	3.1	HEMA and lactic acid

From the results shown in Table 4, it follows that:

1. HEMA-dilactate has a circa 10 fold lower stability than HEMA-monolactate. The fact that the degradation product of HEMA-dilactate is almost exclusively HEMA (and not a mixture of HEMA-monolactate and HEMA) demonstrates that the ester bonds in HEMA-dilactate do not have the same susceptibility for hydrolysis. A possible explanation is that the OH-end group participates via a so called back-biting mechanism in the degradation of HEMA-dilactate yielding HEMA and lactide. The latter compound is then in a two step process rapidly converted into lactic acid.

2. Since hardly any MAA (methacrylic acid) is detected, this demonstrates that the lactate ester bonds are more susceptible for hydrolysis than the methacrylate ester group in HEMA-mono/di-lactate. Further, it is expected that after polymerization of HEMA-mono/di-lactate the susceptibility of the lactate ester group will not differ substantially as compared with

monomers. The length of the lactate graft is therefore an important parameter to control and tailor the time required for an insoluble copolymer with hydrolyzable side groups to become soluble under physiological conditions. Again, this insoluble-soluble conversion will occur when the LCST of the
5 copolymer passes body temperature due to hydrolysis of side groups.

From this Example it follows that the present invention provides a method for controlling the kinetics of the insoluble/soluble conversion of polymers which are grafted with lactate groups, which method comprises changing the DS of said polymer. For example, when the DS is changed from 1
10 to 2, the rate of degradation of the polymer decreases dramatically, i.e. with about a factor ten.

EXAMPLE 10

Degradation of N-(2-hydroxypropyl) 15 methacrylamide-mono(di) lactate (HPMAAm- mono(di)lactate)

The hydrolytic degradation of HPMAAm-monolactate and HPMAAm-dilactate was evaluated essentially as described for HEMA-
20 monolactate and HEMA-dilactate (Example 9). To accelerate the hydrolysis, the samples were incubated in 100mM carbonate buffer, pH 9.0 at 37°C. The degradation of HPMAAm-monolactate was followed for 13 hours and the degradation of HEMA-dilactate was followed for 90 minutes. The different samples were analysed by HPLC (LiChrosphere 100 RP-18 (5 µm, 125 × 4 mm
25 i.d.) to monitor the concentrations of HPMAAm-dilactate, HPMAAm-monolactate, HPMAAm and MMAc (methacrylic acid).

A gradient system was used with methanol / water = 5 /95 (w/w), pH 2 (eluent A) and methanol / water = 95 / 5 (w/w), pH 2 (eluent B). The pH of both eluents was adjusted with perchloric acid. The gradient was run from 100

% A to 100 % B in 26 minutes, with the flow rate 1 ml/min. For detection was used UV-detector at the wavelength 210 nm.

The values of k_{obs} and the half-life ($t_{1/2}$) for HPMAAm-monolactate and HPMAAm-dilactate are presented in Table 5.

5

Table 5.

Monomer	k_{obs} (s ⁻¹) at pH 9.0	$t_{1/2}$ (min) at pH 9.0	$t_{1/2}$ (hrs) at pH 7.5**	Major degradation products
HPMAAm-monolactate	7.5E-05*	154	81	HPMA and lactic acid
HPMAAm-dilactate	5.4E-04*	21	11	HPMAAm, HPMAAm-monolactate and lactic acid

* average of 2 experiments.

** see note 2.

10

From the results shown in this table, it follows that:

1. As was seen in Example 9 for the HEMA-lactate compound, HPMAAm-dilactate is more susceptible to hydrolysis. However, the ester bond which is preferentially cleaved differs. In HEMA-dilactate lactoyl lactate is split off in one step, whereas in HPMAAm-dilactate both lactate and lactoyl lactate are split of.

2. Since hydrolysis of ester in the pH7-9 range is catalyzed by hydroxyl ions, the k_{obs} (and corresponding half-life values) at pH 7.5 can be calculated assuming that the reaction order in OH^- = 1. Both half-lives are however, greater than the half-lives of HEMA-monolactate and HEMA-dilactate (Example 9) which demonstrates that the nature of the hydrolyzable comonomer is therefore an important parameter to control and tailor the time required for an insoluble copolymer with hydrolyzable side groups to become soluble under physiological conditions. Again, this insoluble-soluble conversion will occur when the LCST of the copolymer passes body temperature due to hydrolysis of side groups.

25

EXAMPLE 11**Synthesis of thermosensitive blockcopolymers**

The macroinitiator was synthesized as follows. All glassware was
5 dried in an oven at 150°C for at least one hour. A 50 ml round bottom flask
was loaded with 0.4 mmol polyethylene glycol 5000 monomethylether (PEG
5000), 0.2 mmol of 4,4-azobis(4-cyanopentanoic acid) (ABCPA), 0.06 mmol 4-
(dimethylamino)pyridinium-4-p-toluenesulfonate (DPTS) and 0.6 mmol N,N'-
dicyclohexylcarbodiimide (DCC). The flask was evacuated three times and
10 filled with nitrogen. The flask was then closed with a septum and 3 ml of dry
solvent dichloromethane/N,N-dimethylformamide (DMF) = 1 /1) was added
using syringe. The mixture was stirred at room temperature for 24 hours.
Next, the reaction mixture was filtered, the formed solid (dicyclohexyl urea)
was washed with dichloromethane and the combined organic solutions were
15 evaporated under reduced pressure. Then the product was extracted with
toluene to remove DPTS, the solvent was evaporated, and a second extraction
was performed with diethyl ether to remove any remaining dicyclohexyl urea.

The solid product obtained was dissolved in water and the solution
was filtered to remove any remaining solid particles. The clear and
20 homogeneous aqueous solution was frozen and the macroinitiator was collected
after freeze-drying.

The product was analysed by ¹H-NMR in CDCl₃ and the ratio of
PEG 5000 to ABCPA was about 2:1.

The macroinitiator was further characterized by GPC. To this end,
25 0.144 g of the PEGylated macroinitiator and 0.001 g of 4-methoxyphenol were
dissolved in 5 ml of freshly distilled 1,4-dioxane. 50 µl was diluted with 9.95
ml dichloromethane (HPLC-grade). The solution of the macroinitiator was
heated for at 80°C for 24 hours. Next, 50 µl was diluted with 9.95 ml
dichloromethane. The samples were analysed by GPC (Waters 60F model
30 gradient pump, Waters 410 differential refractometer, Shodex KF 80M column

with Shodex KF-G precolumn). Filtrated dichloromethane was used as the mobile phase; the flow rate was 1 ml/min. The analysis was conducted at 35°C. Polystyrene standards of known molecular weights were used as calibration standards. Table 6 shows the results.

5

Table 6.

Sample	M _n	M _w
PEG 5000	7149	9052
PEG 5000-ABCPA	12587	23496
PEG 5000-ABCPA*	8828	12701

M_n = number average molecular weight,

M_w = weight average molecular weight

10 * after 24 hrs at 80°C

The results from Table 5, combined with the NMR data, clearly demonstrate that a PEGylated macroinitiator has been formed: after the coupling reaction, the molecular weight of formed product is almost doubled;
15 the initiator is decomposed after incubation at 80°C.

The obtained macroinitiator was used to synthesize an AB blockcopolymer with NIPAAm. Therefore, 0.4 g NIPAAm and 0.144 g of the PEGylated macroinitiator (PEG 5000)₂-ABCPA (NIPAAm : ABCPA = 250 : 1 (mol/mol)) were dissolved in 5 ml of freshly distilled 1,4-dioxane. The
20 copolymerization was conducted at 80°C for 24 h in a nitrogen atmosphere. Subsequently, the solvent was removed under reduced pressure and the copolymers were dissolved in acetone (around 20% (w/v)) and precipitated in an excess of diethyl ether. The precipitated polymers were isolated by filtration and dried in a vacuum oven at 40°C. NMR analysis showed that the
25 M_n of the NIPAAm block is 12500 assuming that all PEG chains acted as initiator.

The micelle forming properties the obtained blockcopolymer were investigated using DLS (Dynamic Light Scattering). At room temperature a mixture of water and blockcopolymer (1 mg/ml) gave a clear and homogeneous

solution. After incubation at 37°C (above the LCST of the NIPAAm block), a slightly turbid solution was obtained. DLS measurements revealed that the sizes of the formed micelles were about 150 nm. By lowering the temperature to 25°C, the solution became clear again, demonstrating the reversibility of the
5 micelles. When a control experiment with PNIPAAm was carried out, visible inspection as well as DLS measurements showed the presence of large aggregates at 37°C.

This Example demonstrates the synthesis route via a macroinitiator by the copolymerization reaction with NIPAAm. This route is not limited to
10 NIPAAm, but can easily be extended to different copolymerizations (*e.g.* copolymerization of NIPAAm and HEMA-mono/dilactate (see *e.g.* Example 1) or HPMAM-mono/dilactate (see *e.g.* Example 6)).

Claims

1. Temperature sensitive polymer having a lower critical solution temperature that changes during incubation in an aqueous solution or medium.
2. Polymer according to claim 1 wherein the hydrophilicity changes by
5 incubation in an aqueous solution or medium.
3. Polymer according to claim 1 or 2 in which the polymer is comprised of at least one monomer selected from the group consisting of acrylamide, methacrylamide, acrylic acid, *N*-isopropyl acrylamide (NIPAM), 2-hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA),
10 acrylamide (AAM), glyceryl methacrylate or glycidyl methacrylate (GMA), glyceryl acrylate or glycidyl acrylate (GA), hydroxypropyl methacrylamide (HPMAM), dimethyl-aminoethyl methacrylate (DMAEMA) and dimethylaminoethyl acrylate (DMAEA), and derivatives and substituted species thereof, part of which monomers having an enzymatically or chemically
15 hydrolysable group.
4. Polymer according to any of the previous claims which is a copolymer or a terpolymer of two or more monomers selected from said group.
5. Polymer according to any of the previous claims which is a block copolymer or terpolymer, a random copolymer or terpolymer, or a polymeric
20 network and which polymer is optionally grafted.
6. Polymer according to any of the previous claims which comprises hydrolysable groups, each said hydrolysable group being selected from the group consisting of ester, amide, carbonate, carbamate, and anhydride groups.
7. Polymer according to any of the previous claims which polymer is
25 selected from the group consisting of a copolymer of *N*-isopropyl acrylamide and 2-hydroxyethyl methacrylate-mono-lactate; a copolymer of *N*-isopropyl acrylamide and glyceryl methacrylate; a terpolymer of *N*-isopropylacrylamide

(NIPAAm), 2-hydroxyethyl methacrylate-monolactate and acrylamide; and mixtures thereof.

8. Polymer according to any of the previous claims having a lower critical solution temperature before incubation below mammalian body temperature and a lower critical solution temperature after incubation above mammalian body temperature.

9. Controlled release system comprising a temperature sensitive polymer according to any of the previous claims and an active ingredient.

10. Controlled release system according to claim 9 in which the polymer is in the form of a polymeric micelle in which a hydrophilic block is present which preferably comprises poly(ethyleneglycol).

11. Targeting drug comprising a drug and particles of a controlled release system according to claim 9 or 10, which particles have an average diameter of less than 100 nm.

12. Targeting drug according to claim 11 which comprises a homing device.

13. 2-(Methacryloyloxy)ethyl-lactate and oligolactates thereof, preferably 2-(methacryloyloxy)ethyl-dilactate.

14. Copolymer of N-isopropyl acrylamide (NIPAAm) / hydroxypropyl methacrylamide lactate (HPMAm-lactate).

15. Copolymer according to claim 14, wherein the ratio of NIPAAm / HPMAm-lactate is from 5-80, preferably from 20-50.

16. Process for the preparation of a temperature sensitive polymer comprising synthesizing a water soluble polymer by a) functionalizing a monomer with hydrolysable groups, b) mixing of said monomer with at least one monomer of a different type using a suitable solvent in the presence of an initiator and/or a catalyst to form said polymer c) removing said solvent and dissolving the polymer, and d) precipitating said polymer; in which process the functionalizing of the monomers of step a) is optionally carried out after step b)

on the monomer units as incorporated in the polymer chain; and subsequently mixing said water soluble polymer with a releasable compound.

17. AB block copolymer, which forms micelles when the lower critical solution temperature (LCST) is passed.
- 5 18. ABA block copolymer, which forms a hydrogel when the lower critical solution temperature (LCST) is passed.
19. Method for controlling the solubility of polymers which are grafted with lactate groups in a certain degree of substitution (DS), which method comprises changing the DS of said polymer.
- 10 20. Use of hydrolysable chemical groups in a temperature sensitive polymer in a controlled release system comprising a releasable compound in order to change said polymer's solution characteristics after incubation.

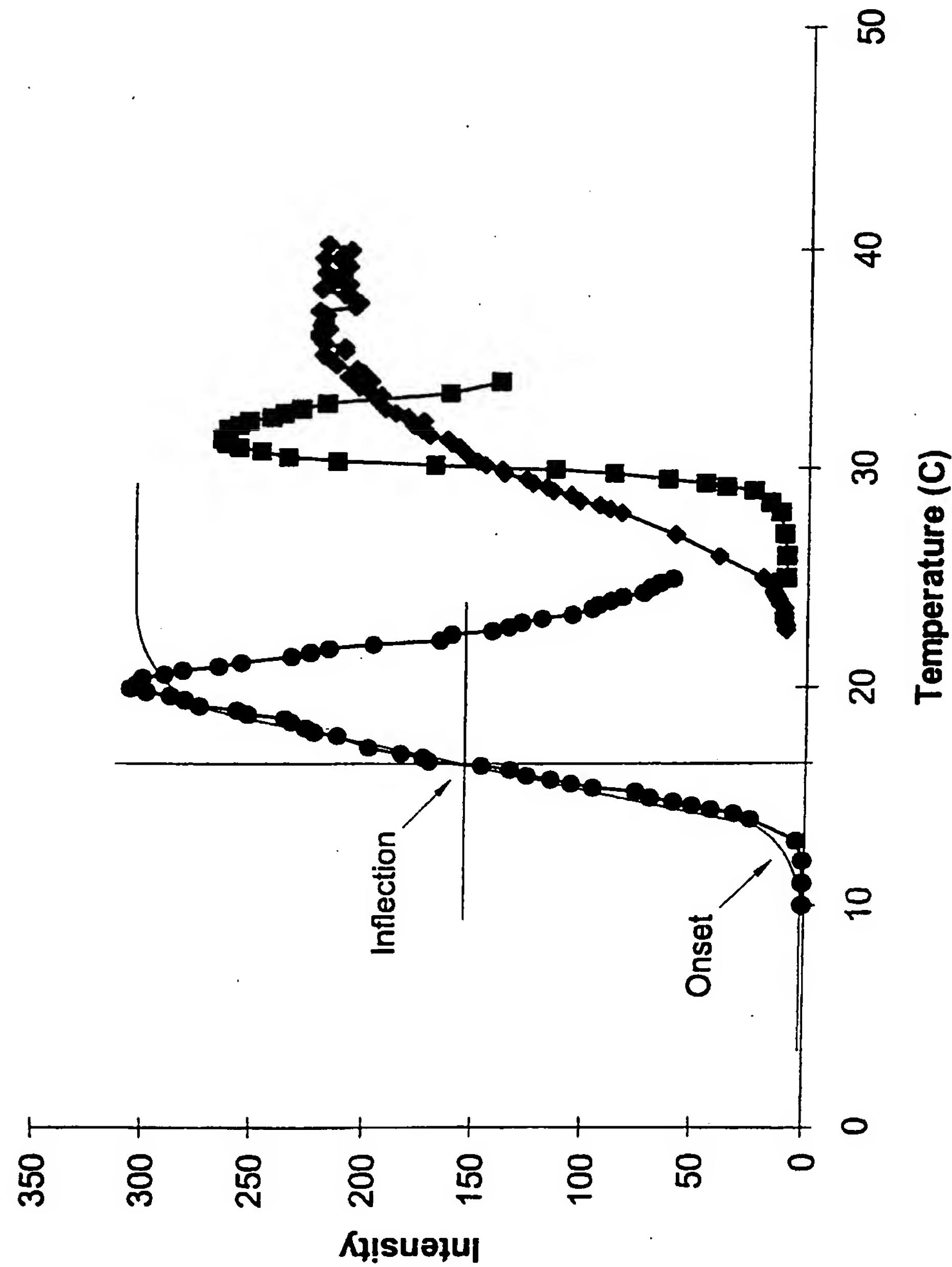


Fig. 1

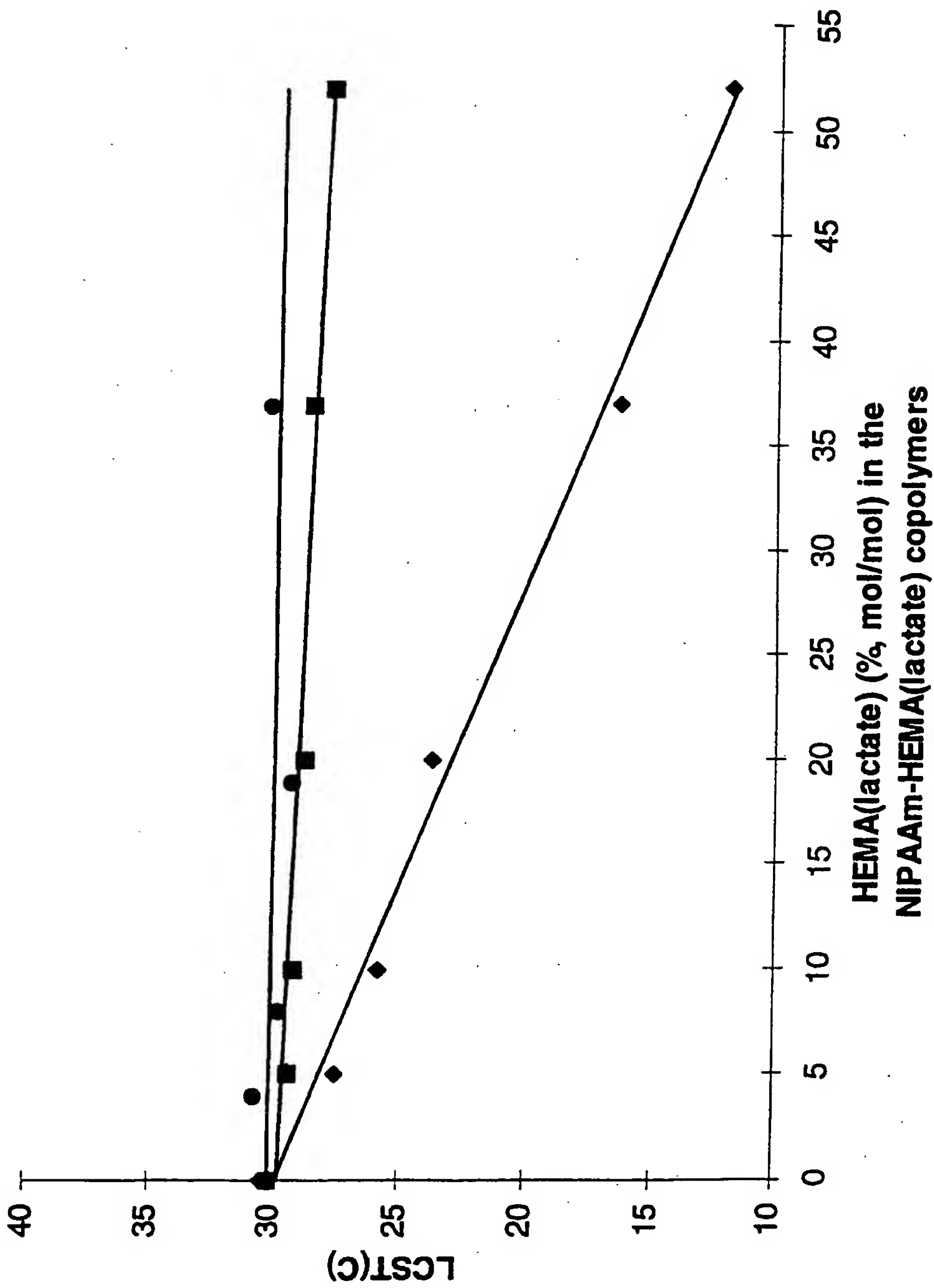


Fig. 2

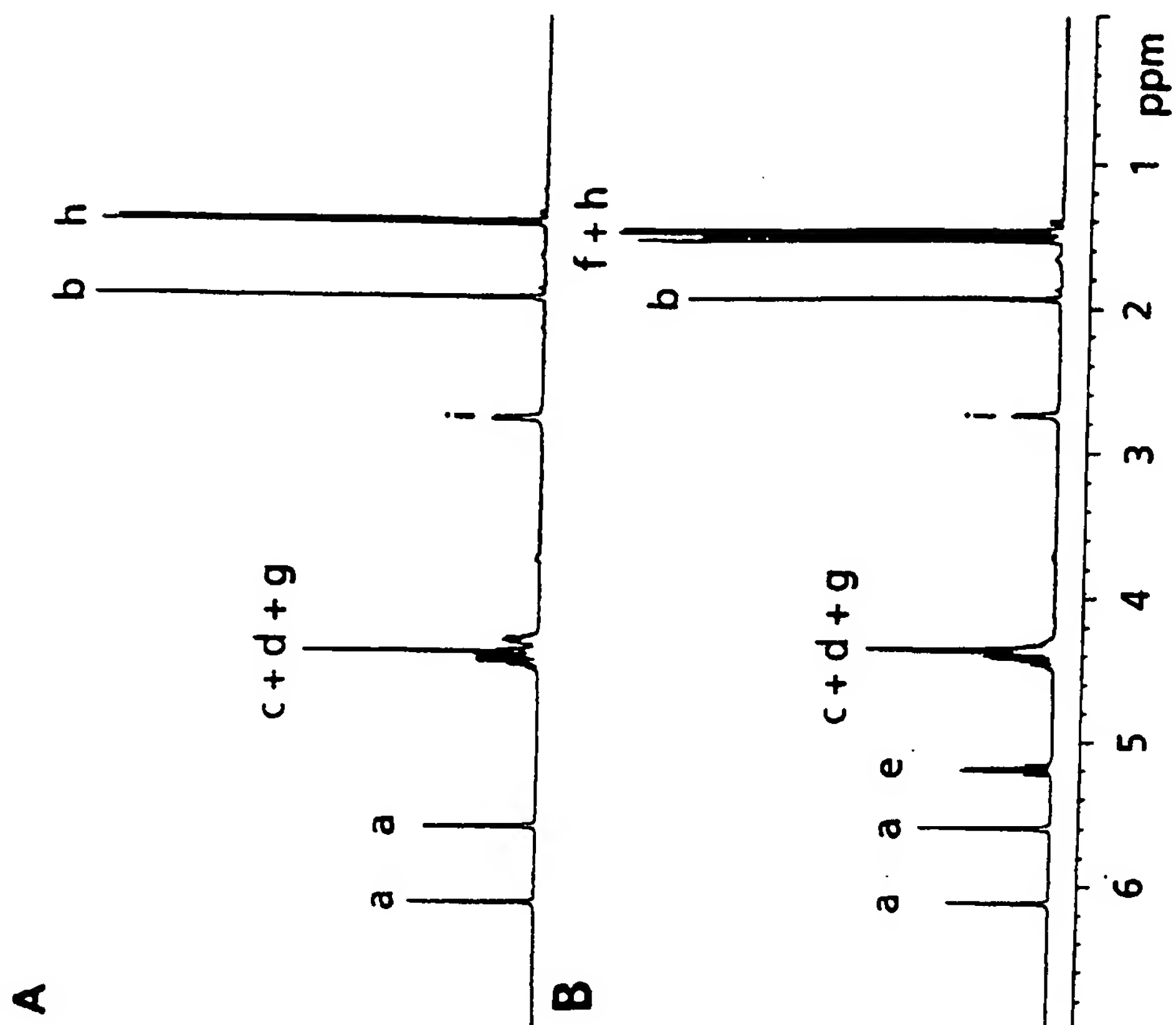
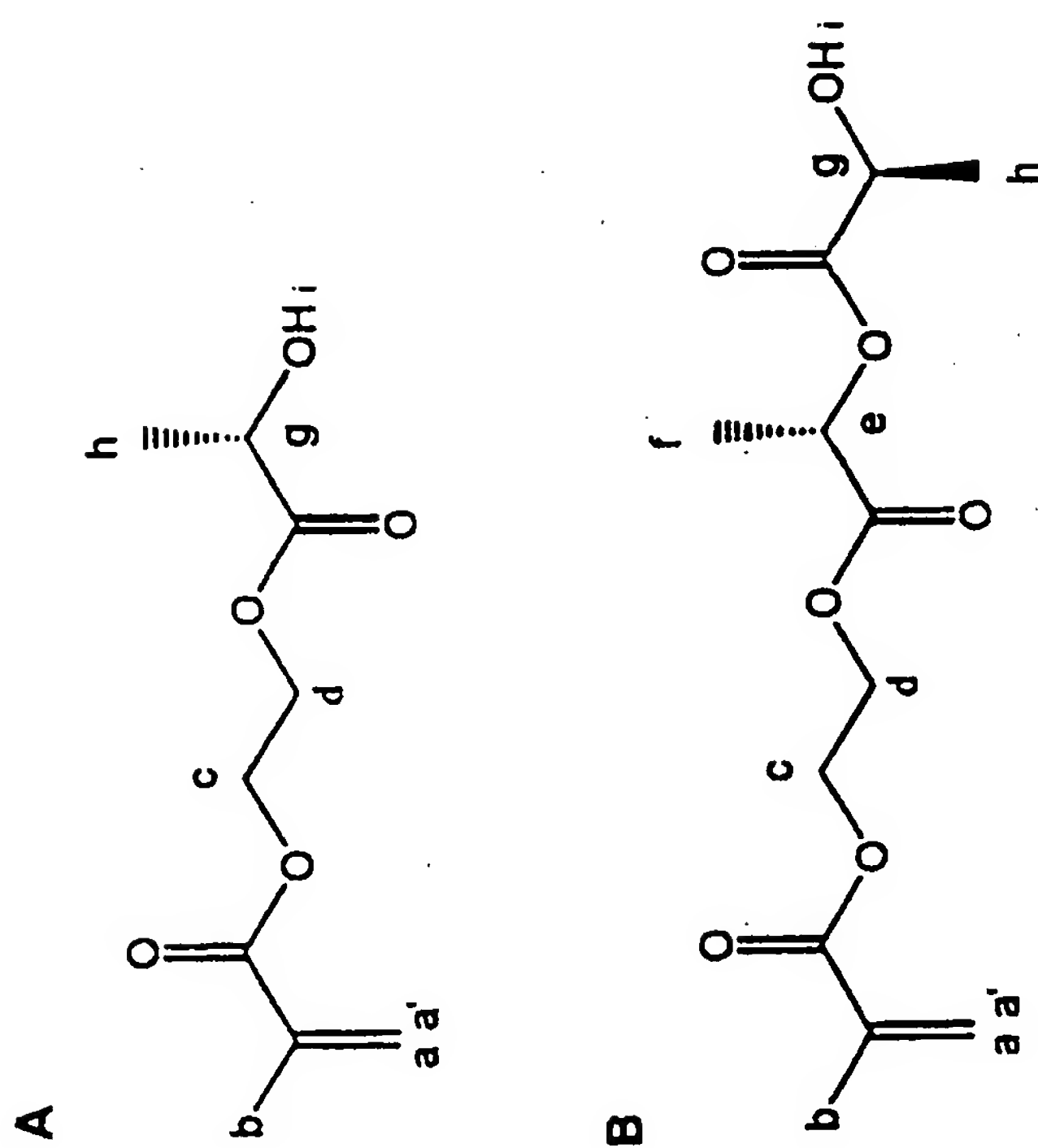


Fig. 3

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/NL 00/00542

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08F8/12 C08F220/54 C08F220/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08F A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

PAJ, WPI Data, EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 720 976 A (JONG-DUK KIM) 24 February 1998 (1998-02-24) the whole document ---	1-20
Y	EP 0 693 508 A (KOHJIN CO., LTD.) 24 January 1996 (1996-01-24) page 2, line 50 -page 3, line 25 page 3, line 55 -page 4, line 20; claims 1-9 ---	1-20
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Y	WO 92 07881 A (MARATHON OIL COMPANY) 14 May 1992 (1992-05-14) claims 1-18; example 3 ---	1-20
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

31 October 2000

Date of mailing of the international search report

08/11/2000

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INTERNATIONAL SEARCH REPORT

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